

HOSTED BY



Contents lists available at ScienceDirect

## HAYATI Journal of Biosciences

journal homepage: <http://www.journals.elsevier.com/hayati-journal-of-biosciences>

Original research article

Marine Microalgae *Tetraselmis suecica* as Flocculant Agent of Bio-flocculation MethodMujizat Kawaroe,<sup>1,2\*</sup> Tri Prartono,<sup>1</sup> Adriani Sunuddin,<sup>1</sup> Deni Saputra<sup>1</sup><sup>1</sup> Department of Marine Science and Technology, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia.<sup>2</sup> Surfactant and Bioenergy Research Centre, Bogor Agricultural University, Bogor, Indonesia.

## ARTICLE INFO

## Article history:

Received 24 September 2014

Accepted 29 September 2015

Available online 19 July 2016

## KEYWORDS:

bio-flocculation,  
harvest,  
microalgae,  
*Tetraselmis suecica*

## ABSTRACT

Microalgae harvesting is an important part in microalgae cultivation system. Techniques for harvesting marine microalgae which are commonly used are centrifugation, filtration and flocculation. These techniques still have some disadvantages, such as not environment friendly, and high usage of energy and cost. Bio-flocculation harvesting technique using microalgae as a flocculant agent can be an alternative way to solve these problems. In this research, mixing of *Tetraselmis suecica* (flocculant) with *Chlorella* sp. and *Nannochloropsis* sp. (non-flocculant) in ratios of 1:4, 2:4, 3:4 and 4:4 (v/v) has been conducted to obtain percent recovery of marine microalgae harvest. The results showed that *T. suecica* as flocculant agent can fasten the harvesting of *Chlorella* sp. and *Nannochloropsis* sp. It was shown by the increase of percent recovery value of *Chlorella* sp. from  $51.14 \pm 1.07\%$  to  $67.34 \pm 0.67\%$  and *Nannochloropsis* sp. from  $20.52 \pm 1.17\%$  to  $42.43 \pm 0.40\%$  during the first hour of flocculating process. Our result showed that bio-flocculation is an environment friendly technique which can be applied to harvest marine microalgae.

Copyright © 2016 Institut Pertanian Bogor. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Harvesting microalgae is an important part of microalgae cultivation system to produce high biomass (Sim *et al.* 1988). Centrifugation, filtration and flocculation are the most common harvesting technique used in harvesting microalgae (Grima *et al.* 2003; Chen *et al.* 2011). Centrifugation is a microalgae harvesting technique which needs high capital, energy and operating costs (Wijffels and Barbosa 2010). Harvesting microalgae using filtration technique can only be performed for microalgae species that are larger than 100  $\mu\text{m}$  in size and filaments or colonized body shape, such as *Spirulina* sp. and *Micractinium* sp. (Mohn 1988). Harvesting microalgae smaller than 100  $\mu\text{m}$  and not colonized can be done using flocculation. Microalgae flocculation technique will concentrate target by using chemical material (Lee *et al.* 1998; Papazi *et al.* 2010), bacteria (Choi *et al.* 1998; Fujita *et al.* 2000; Salehizaden *et al.* 2000; Li and Yang 2007) and fungi (Chang *et al.* 2005).

Harvesting microalgae using chemical flocculant is very easy to be done, but not suitable for large scale cultivation because the

excessive cationic flocculants must be cleaned from the culture media so it can be re-used (Schenk *et al.* 2008). Flocculation using chemicals can also change the culture media conditions such as extreme pH changes, nutrient decline, and changes in temperature and dissolved oxygen. The addition of chemical compounds can also cause changes in microalgae cell composition (Benemann and Oswald 1996). Flocculation using living organisms (bio-flocculation) can be used as the alternative, so the chemical contamination effect can be reduced.

The use of fungi and bacteria as flocculants requires particular additional media as the energy source for growth. Furthermore, bacteria and fungi can cause contamination of microalgae (Schenk *et al.* 2008). Salim *et al.* (2011) stated that bio-flocculation technique using microalgae is more promising than bacteria and fungi because it does not require additional operating costs for growth media and also prevents contamination. The most important thing from using microalgae for bio-flocculation is it is environment friendly because it does not use chemicals and the cultivation media can be re-used.

Bio-flocculation technique can be used as an alternative method for microalgae harvest. It is urgent to know an optimal ratio of flocculant microalgae to harvest non-flocculant microalgae. Salim *et al.* (2011) conducted a bio-flocculation research of marine

\* Corresponding author.

E-mail address: [mujizat@gmail.com](mailto:mujizat@gmail.com) (M. Kawaroe).

Peer review under responsibility of Institut Pertanian Bogor.

microalgae species using *Tetraselmis suecica* as flocculant microalgae and *Neochloris oleoabundans* as non-flocculant microalgae. In this study, *T. suecica* was used as flocculants and *Nannochloropsis* sp. and *Chlorella* sp. as non-flocculant. The reason for selecting *Nannochloropsis* sp. and *Chlorella* sp. is they are easy to cultivate in short cultivation time and potential as a source of biofuel since they have high fat content.

The purpose of this study was to obtain the optimal harvest time, determine ratio of volume between flocculant (*T. suecica*) and non-flocculant microalgae (*Nannochloropsis* sp. and *Chlorella* sp.) to improve microalgae deposition and measure their fat content.

## 2. Material and Methods

### 2.1. Microalgae and culture conditions

Marine microalgae *T. suecica*, *Nannochloropsis* sp. and *Chlorella* sp. were obtained from Microalgae Laboratory at Surfactant and Bioenergy Research Center, LPPM-IPB, Baranangsiang Campus, Bogor, Indonesia. Microalgae strain was cultivated at 500 mL for each species. The strain multiplication was done by cultivating microalgae from 500 mL to 1.5 L and from 1.5 L to 4.5 L for each species. Volume composition of microalgae and seawater in laboratory scale was one third, while composition of scale up at outdoor system was one tenth (Kawaroe *et al.* 2010). Nutrient used at laboratory scale was Walne with composition 1 mL Walne to 1 L microalgae medium, while at outdoor scale, TSP, ZA, urea with composition of 15, 30, 30 ppm, respectively.

### 2.2. Determination of harvest time

Harvest time of each microalgae species might be different so it needs pre-research to determine the optimal harvest time. *T. suecica*, *Nannochloropsis* sp. and *Chlorella* sp. were taken each for 300 mL and divided equally into three bottles (100 mL), and then 200 mL of autoclave-sterilized seawater was added. The cultivation was conducted for 20 days with aeration. During 20 days of cultivation, microalgae cell density was measured using Neubauer hemocytometer under microscope type BM180 BOECO Germany. The highest density of microalgae then became the reference time for harvesting microalgae so that the first day of cultivation at bio-flocculation process can be determined.

### 2.3. Bio-flocculation process

Bio-flocculation process was conducted to determine optimal ratio of flocculant volume to settled non-flocculant microalgae and also bio-flocculation mechanism so non-flocculant microalgae can be settled faster. Bio-flocculation research takes less than 1 day to prevent cell splitting. Cultivation results of *T. suecica*, *Nannochloropsis* sp. and *Chlorella* sp. were stored in refrigerator before the flocculation process has begun. Ratios of mixing flocculant and non-flocculant microalgae were 1:4, 2:4, 3:4 and 4:4 (v/v) in 2 mL. Furthermore, optical density (OD) 750 nm measured every hour during 8 hours or until the perfect settlement occurs between flocculants and non-flocculant microalgae. Calibration process was done using blank sample of seawater. OD750 nm values were obtained to determine percentage of microalgae deposition (recovery). Spectrophotometer with single beam Genesys 20 4001/4 Model with wavelength range of 340–800 nm was used. Bio-flocculation process was done using Olympus microscope CX21LED. Photos and videos were taken by Optilab Microscope Camera. This observation was done in conjunction with OD750 nm value observation. Image capture was done at  $t_0$  (time 0),  $t_4$  (time 4) and  $t_8$  (time 8) whereas video capture was done until the mixture settles microalgae perfectly.

### 2.4. Microalgae lipid extraction

Lipid extraction requires dried microalgae in greater numbers so that requirement of bio-flocculation process was 10 L. Lipid value from bio-flocculation was compared to harvesting results using NaOH. Microalgae paste from bio-flocculation and NaOH harvesting was dried using oven at 121°C for  $\pm 24$  hours. Dried microalgae were then transferred to desiccator for  $\pm 15$  minutes. After being cooled in the desiccator, dried microalgae were measured using analytical balance Precisa. Microalgae lipid was extracted using Soxhlet in n-hexane solvent. Extraction process was conducted for 6 hours and followed by distillation process to separate lipid from solvent. The end result was microalgae lipid weighed and lipid percentage was measured.

### 2.4. Data analysis

- Density cells: Microalgae cell density was calculated using formula improved Neubauer hemocytometer:

$$N_i = n_i \times \left(\frac{25}{5}\right) \times 10^4 \quad (1)$$

$N_i$  is microalgae cell density in the  $i$ -th observation box (number of cells/mL) and  $n_i$  is number of microalgae cells in the  $i$ -th observation box.

- Deposition: Microalgae removal percentage/deposition (percent recovery) was obtained from OD750 and calculated with formula: (Salim *et al.* 2011)

$$\text{Deposition (\%)} = \frac{\text{OD}_{750}(t_0) - \text{OD}_{750}(t_n)}{\text{OD}_{750}(t_0)} \times 100\% \quad (2)$$

OD750 time 0 ( $t_0$ ) is the turbidity in the beginning bio-flocculation process and OD750 time  $n$  ( $t_n$ ) is the turbidity at time  $n$ .

- Lipid content: Microalgae lipid was calculated using the equation below.

$$\text{lipid content (\%)} = \frac{A}{B} \times 100\% \quad (3)$$

A is microalgae dry weight (gr) and B is microalgae lipid weight (gr)

- Statistical analysis: completely randomized design with two factors was used to determine the effect of the microalgae volume ratio used in flocculation process. Process was done separately for each combination of microalgae with ratios of 1:4, 2:4, 3:4 and 4:4 to find out the best combination between *T. suecica* and *Chlorella* sp. and also between *T. suecica* and *Nannochloropsis* sp. in flocculation process, so that midvalue test was examined.

## 3. Results

### 3.1. Microalgae harvest time

Based on cell density observation in 20 days, the best time to harvest *T. suecica* was at day 13 with cell density of  $1.98 \times 10^6 \pm 0.49 \times 10^6$  cells/mL (Figure 1). *Chlorella* sp. was found at day 12 with cell density  $169.17 \times 10^6 \pm 15.93 \times 10^6$  cells/mL, whereas *Nannochloropsis* sp. grew faster than *T. suecica* and *Chlorella* sp. which was at day 11 with cell density  $995.42 \times 10^6 \pm 216.89 \times 10^6$  cells/mL.

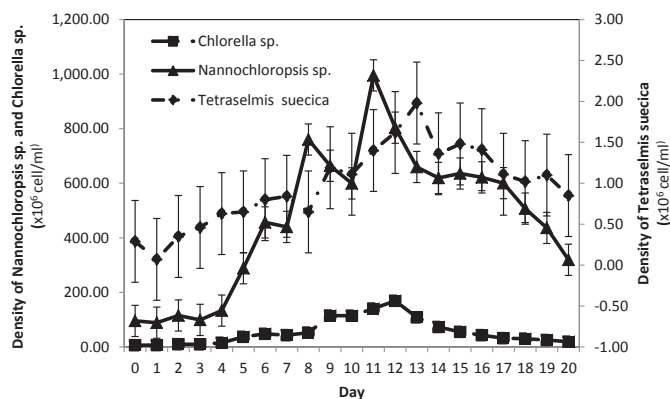


Figure 1. Growth phase of *Tetraselmis suecica*, *Chlorella* sp., and *Nannochloropsis* sp.

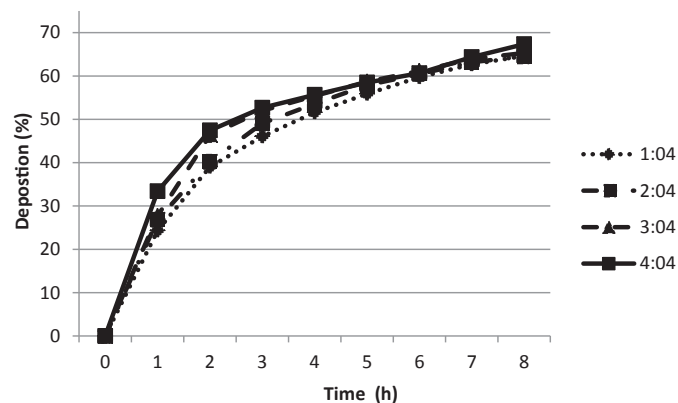


Figure 2. Deposition (%) of *Tetraselmis suecica* and *Chlorella* sp. combination with ratios of 1:4, 2:4, 3:4 and 4:4.

### 3.2. Bio-flocculation microalgae

Recovery percentage of microalgae as results of bio-flocculation in the beginning with volume ratios of 1:4, 2:4, 3:4, 4:4 can be found in Table 1. Statistical analysis using completely randomized design implied that volume of 4:4 was the most optimal ratio compared to 1:4, 2:4 and 3:4. It is clear that the addition of flocculant species in larger volume will increase deposition percentage. A graph showing deposition percentage (OD750 nm) of microalgae in mixed combinations of *T. suecica*:*Chlorella* sp. and *T. suecica*:*Nannochloropsis* sp. with ratio of 1:4, 2:4, 3:4 and 4:4 in every hour during 8 hours can be seen in Figures 2 and 3. Bio-flocculation of microalgae before flocculate (time 0) and after flocculate (time 4 and 8) can be seen at Figure 4. Observation of bio-flocculation process for *T. suecica* and *Chlorella* sp. or *T. suecica* and *Nannochloropsis* sp. with 10 $\times$  magnification showed that cells of *Chlorella* sp. and *Nannochloropsis* sp. were bound to local form of *T. suecica*, not in large network form that are connected to one another (bridging; Figures 4A and 4B). At the beginning of mixing, microalgae flocculants and non-flocculant cell were still separated and not tied at all (Figures 4C and 4D). Gradually, the bonding process began to take place as cells began to experience stress due to diminishing nutrients and triggered to secrete extracellular polymer. Extracellular polymer production will create a bond between cells centered in *T. suecica*. The bond between flocculant and non-flocculant microalgae cells started at  $t_4$  (Figures 4E and 4F) and can be seen from bond formation between species. More bounding began to be seen at  $t_8$  (Figures 4G and 4H) and was clearly visible microscopically for non-flocculant species. It formed a bigger colony with flocculant species, causing a lot of colony which then settles.

### 3.3. Microalgae lipid from bio-flocculation harvesting process

Harvesting was done using 4:4 volume ratio between microalgae flocculants and non-flocculant. For comparison, harvesting microalgae using NaOH was conducted. Lipid extraction results obtained using Soxhlet are provided in Table 2. Extraction of microalgal lipid from bio-flocculation techniques using *T. suecica* produced higher lipid content compared to chemical flocculation techniques using NaOH.

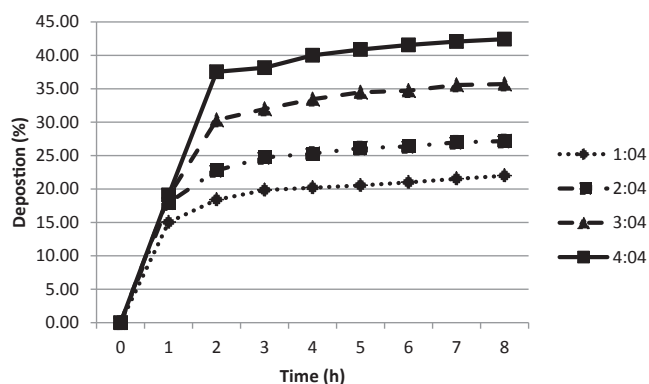


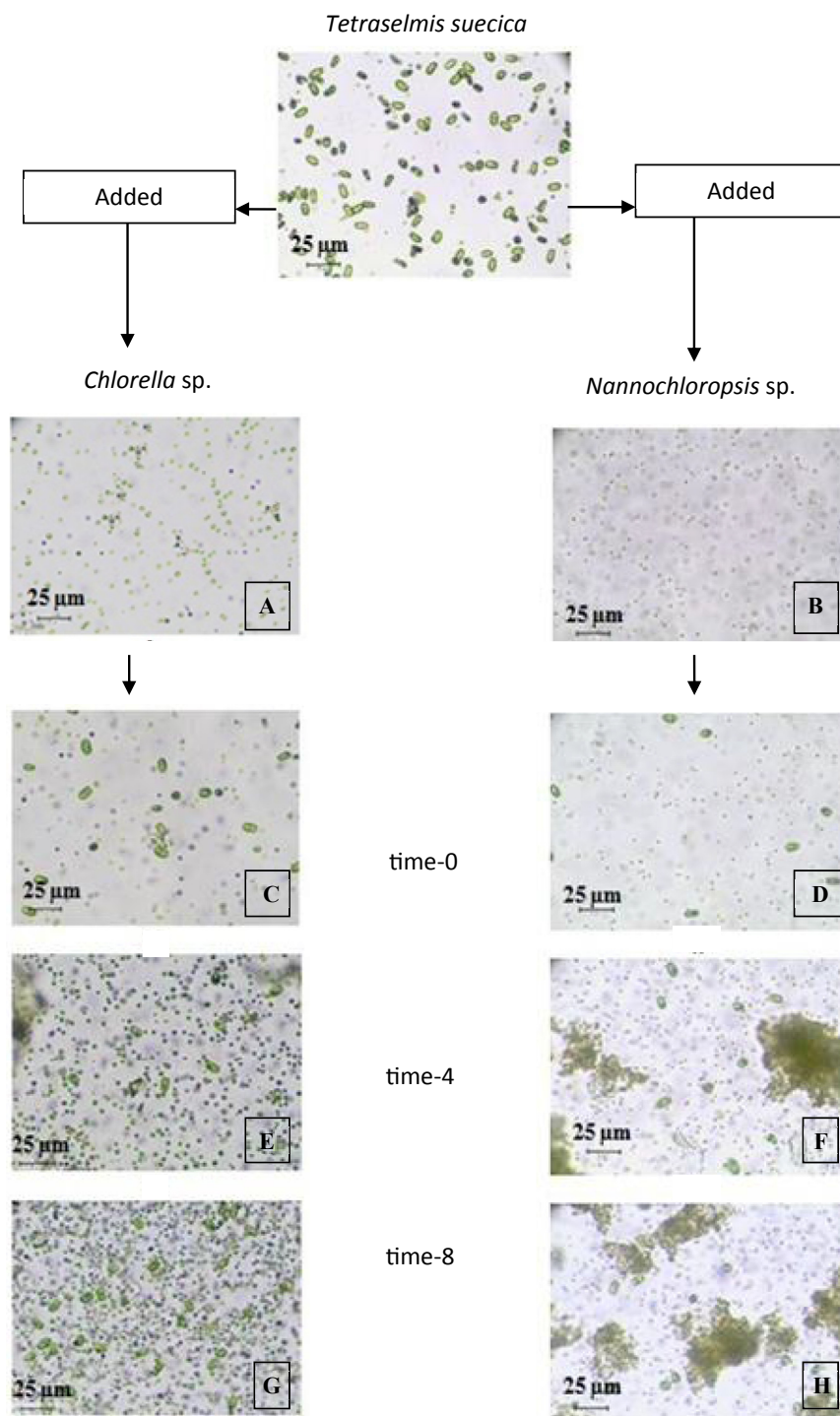
Figure 3. Deposition (%) of *Tetraselmis suecica* and *Nannochloropsis* sp. with ratios of 1:4, 2:4, 3:4 and 4:4.

## 4. Discussion

Lee et al. (1998) mentions that bio-flocculation is a spontaneous flocculation of microalgae cells that occurs due to the secretion of extracellular polymeric substances (EPS) when microalgae is in stress state. Lack of nutrition is a major factor that causes microalgae cell to secrete EPS (Lee et al. 2009). EPS produced by microalgae will trigger the formation of cells clumps, the cells will form biomass collection and then will be sedimented. Microalgae harvesting by bio-flocculation techniques using flocculation agents in the form of microalgae is a new harvesting technique which is still in development stage. Bio-flocculation technique has some advantages: it is environment friendly where residual water from harvesting can be used and disposed without any special treatment that can add to the cost and energy in microalgae processing chain into biofuels. The use of chemicals can contaminate water for microalgae cultivation. The remaining water after harvest when discharged directly into the environment must be processed first to avoid contamination. This process requires quite much energy and cost. Our results showed that microalgae harvesting by

Table 1. Deposition (%) of microalgae flocculant and non-flocculant mixing after first hour

Microalgae combination	Deposition (%) time 1			
	A	B	C	D
<i>Tetraselmis suecica</i> & <i>Chlorella</i> sp.	24.28 $\pm$ 4.55	26.83 $\pm$ 1.30	27.70 $\pm$ 3.98	33.40 $\pm$ 1.17
<i>Tetraselmis suecica</i> & <i>Nannochloropsis</i> sp.	15.02 $\pm$ 1.33	17.95 $\pm$ 1.95	18.95 $\pm$ 0.88	19.11 $\pm$ 0.22



**Figure 4.** Microalgae before flocculate (time 0) and after flocculate (time 4 and 8).

**Table 2.** Comparison of lipid yields from bio-flocculation and chemical harvesting method

Microalgae	Harvesting agent	Lipid content (%)
<i>Nannochloropsis</i> sp.	NaOH	10.29 ± 3.16
<i>Nannochloropsis</i> sp.	<i>Tetraselmis suecica</i>	12.90 ± 2.62
<i>Chlorella</i> sp.	NaOH	8.99 ± 1.71
<i>Chlorella</i> sp.	<i>Tetraselmis suecica</i>	11.71 ± 0.81

bio-flocculation technique using microalgae flocculation agent can be done. It can be seen from the deposition percentage of microalgae non-flocculant was increased for both species; *Chlorella* sp. and *Nannochloropsis* sp. Comparison between chemical and bio-flocculation technique showed that the latter is better than the former. The difference is because chemical compounds that are used do not contain fat, in contrast to the use of *T. suecica* as flocculant agent which has 15%–23% of lipid content (Chisti 2007). The lipid from *T. suecica* can also be extracted along with species target



Table 3. Deposition (%) comparison before and after addition of species flocculant within 8 hours

Species	Deposition (%) within 8 hours	
	Before adding <i>Tetraselmis suecica</i>	After adding <i>Tetraselmis suecica</i> <sup>a</sup>
<i>Chlorella</i> sp.	51.14 ± 1.07	67.34 ± 0.67
<i>Nannochloropsis</i> sp.	20.52 ± 1.17	42.43 ± 0.40

<sup>a</sup> Ratio 4:4.

(*Nannochloropsis* sp. and *Chlorella* sp.). Utilization of microalgae as flocculant agent gives a distinct advantage because lipid from flocculant microalgae can be extracted as well.

Our results suggest that microalgae harvesting using bio-flocculation technique can be used as an environment friendly harvest and alternative way to substitute chemical flocculants. In addition, it can also increase lipid content because of the addition of microalgae flocculant (Table 3). Bio-flocculation process during 8 hours has not yet successfully settled microalgae 100%. This happens because *T. suecica* cell density was much lower than *Chlorella* sp. and *Nannochloropsis* sp. so that upon mixing process, *T. suecica* as the center of collecting *Chlorella* sp. and *Nannochloropsis* sp. was unable to bind *Chlorella* sp. and *Nannochloropsis* sp. as a whole because cell number of *Chlorella* sp. and *Nannochloropsis* sp. was much greater. The addition of flocculants density will accelerate deposition process because it will add the centers of cell attachment to non-flocculant formation and aggregates more easily. Furthermore, this harvesting techniques can be used as an alternative to harvest microalgae. Besides eco-friendly, it is also a promising method because it is cheaper and uses less energy than the other techniques (Salim et al. 2011).

Flocculant species *T. suecica* was harvested at day 13, whereas *Nannochloropsis* sp. and *Chlorella* sp. were non-flocculant species with successive harvesting days 11 and 12. Harvesting microalgae using bio-flocculation with volume ratio of flocculant and non-flocculant 4:4 was the most optimal ratio for deposition of microalgae and higher than other ratios. From microalgae lipid extraction result, it was shown that lipid content of *Nannochloropsis* sp. and *Chlorella* sp. harvested using bio-flocculation technique was higher compared to chemical flocculants, which shows that bio-flocculation using microalgae can be used as an alternative way for harvesting microalgae.

## Acknowledgements

The research was financially supported from Operational Support for Public University (BOPTN) – Directorate of Higher Education (DIKT) 2013.

## References

- Benemann JR, Oswald WJ. 1996. Systems and Economic Analysis of Microalgae Ponds for Conversion of CO<sub>2</sub> to Biomass. Final Report. United States.
- Chang YI, Shih LH, Chen SW. 2005. Effects of glucose and mannose on the flocculation behavior of *Saccharomyces cerevisiae* at different life stages. *Colloid Surface B* 44:6–14.
- Chen CY, Yeh KL, Aisyah R, Jong D, Chang JS. 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource Technol* 102:71–81.
- Chisti Y. 2007. Biodiesel from microalgae. *Biotechnol Adv* 25:294–306.
- Choi CW, Yoo SA, Oh IH, Park SH. 1998. Characterization of an extracellular flocculating substance produced by a planktonic *Cyanobacterium*, *Anabaena* sp. *Biotechnol* 20:643–6.
- Fujita M, Ike M, Tachibana S, Kitada G, Kim SY, Inoue Z. 2000. Characterization of a bioflocculant produced by *Citrobacter* sp. TKF04 from acetic and propionic acids. *Biosci and Bioeng* 89:40–6.
- Grima EM, Belarbi EH, Fernandez FGA, Medina AR, Chisti Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* 20:491–515.
- Kwaroe M, Prartono T, Sunuddin A, Sari DW, Agustine D. 2010. Mikroalga Potensi dan Pemanfaatan untuk Produksi Bio Bahan Bakar. Bogor: IPB Press.
- Lee AK, Lewis DM, Ashman PJ. 2009. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *Appl Phycol* 21:559–67.
- Lee SJ, Kim SB, Kim JE, Kwon GS, Yoon BD, Oh HM. 1998. Effects of harvesting method and growth stage on the flocculation of the green algae *Botryococcus braunii*. *Lett Appl Microbiol* 27:14–8.
- Li XY, Yang SF. 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Res* 41:1022–30.
- Mohn FH. 1988. Harvesting of microalgae biomass. In: Borowitzka MA, Borowitzka LJ (Eds.). *Microalgae Biotechnology*. Cambridge University Press. 477.
- Papazi A, Makridis P, Divanach P. 2010. Harvesting *Chlorella minutissima* using cell coagulants. *Appl Phycol* 22:349–55.
- Salehizaden H, Vossoughi M, Alemzadeh I. 2000. Some investigations on bio-flocculant producing bacteria. *Biochem Eng* 5:39–44.
- Salim S, Bosman R, Vermue MH, Wijffels RH. 2011. Harvesting of microalgae by bio-flocculation. *Appl Phycol* 23:849–55.
- Schenk PM, Hall SRT, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy* 1:20–43.
- Sim TS, Goh A, Becker EW. 1988. Comparison of centrifugation, dissolved air flotation and filtration technique for harvesting sewage-grown algae. *Biomass* 16:51–62.
- Wijffels RH, Barbosa M. 2010. An outlook on microalgal biofuels. *Science* 329:296–9.